# CHARACTERIZATION OF ARSENITE OXIDIZING BACTERIA FOR WASTEWATER TREATMENT.

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## 1. RESUMEN.

This work is focuses on the mining town of Xichu, Guanajuato, where water has been contaminated by arsenic (As) since its river passes by the Aurora mine tailings. In the dry season a high concentration of As (98 µg L-1) has been detected, exceeding the limits established by the WHO (10µg L-1) and the Mexican norm NOM-127-SSA1 (25 µg L-1). Currently, due to the lack of economic resources in this rural area cannot be apply a conventional water treatment as chemical and physical methods are. Bioremediation of waters using heavy metal resistant bacteria-based technologies may provide a better alternative. It was statistically corroborated the capacity of 3 bacteria strains to interact with arsenite, showing their possible application during the oxidation process of arsenite or as a bioaccumulation process.

**Palabras clave: Bacteria, Arsenite oxidation, Biological treatment**

## 2. INTRODUCCIÓN

Arsenic (As) contamination in drinking water represents one of the most challenges to human health. Around the world, it is estimated that more than 100 million people are at risk of drinking water contaminated with As (Rodríguez, Serafínb, Canoc, Gutiérrezb, & Álvarezc, 2019). The situation in Latin America is serious, due to the few studies and publications that reflect the real problem about arsenic in water. For instance, in Mexico is estimated that there are around 2 million population exposure to arsenic water contamination(Castrejón, Muñoz, Canchola, & Vargas; Osuna-Martínez, Armienta, Bergés-Tiznado, & Páez-Osuna, 2020). The situation becomes more complex in rural areas, due to the lack of economic resources, in this context, in Mexico is estimated that there are around of 500,000 population in rural communities drinking contaminated water with arsenic, it which is above the WHO (World Health Organization) and Mexican normativity. Despite exist several successfully physicochemical water treatment systems to remove As from water such as, coagulation/filtration, ion exchange, etc. these it cannot be applied in rural communities due to they are very expensive and polluting, for this reason the project have been focused in biological treatments, since they do not produce environmental impacts and they are relatively cheaper. Although the high arsenic

toxicity, some microorganisms can resist high As levels and interact with it by different forms. Microbial As (III)-oxidation is one of the most promising mechanisms, as a precursor step in As removal from contaminated water, since conventional iron-based treatment are more effective in removing As (V) rather than As (III) (Natasha et al., 2019; Xia, Wang, & Wan, 2020). Microbial As (III) oxidation represents a detoxification process in heterotrophic microorganisms as *Herminiimonas arsenicoxydans (Castrejón et al.; Correa-Mendez et al., 2014)*, or an energetic metabolism in chemolithoauthotrophic microorganisms, such as *Thiomonas arsenivorans* (Soto et al., 2019). Both oxidation mechanisms are carried out by the enzyme arsenite oxidase(Xia et al., 2020) .

## 3. METHODOLOGY

### 3.1 Study area.

The study area is located within the state of Guanajuato, Mex. Xichu is the municipally name (Rodríguez et al., 2019). Here, there was mining activity since the sixteenth century, leaving tons of mining waste around the principal body water of the region “the Xichu River”. Which present As concentration above 90 µg L-1 in dry season.

### 3.2 Growth Kinetics.

Growth kinetics in M9 medium with 100 µgL-1 of As(III). Incubation conditions: temperature 30°C at 120 rpm for 8 days. Each assay was measured every 24 hours.

### 3.3 Amplification of aox genes and search for arsenic resistance determinants.

Amplification, PCR was mixed with Water miliQ, Buffer PCR (10X), MgCl2, dNTPs, and the initiators aoxA and aoxB (in the case of arsenite oxidase), and finally DNAg and Taq Polymerase (Invitrogen). The reaction was carried out in a Thermocycler (LabNet), at an alignment temperature of 60 °C, ending with the Extension Phase at 72 ºC for 1 minute. Finally, a cycle was applied for a final extension of 10 min. at 72ºC and finally a maintenance stage was programmed at 4°C.
The extraction of plasmid DNA was carried out by the alkaline lysis method described by Birboim and Doly 1979.

### 3.4 Relation between production of Polyhydroxybutarate (PHB) and the arsenite resistance.

The determination of PHB production was through Sudan Black Staining, and it was used an optic mi-croscope 100X to observe the PHB production with-in the cell. Solution I (Sudan Black 0.33% (w / v) in 60% ethanol) and solution II (Safranin 0.5% (w / v) in aqueous solution).

### 3.5 Statistical analysis.

The growth kinetics tests were performed in duplicate and a statistical analysis of the data was implemented using an ANOVA analysis of a factor, and the Tukey multiple comparisons method (P <0.05 and level of significance of α = 0.05). The above by using the MiniTab 2019 software. This to check the real growth of the strains and identify the differences in their growth in contact with As (III).

## 4. RESULTS.

In table 1 it shows the results. According with the observed in the growth kinetics and with the ANOVA and Tukey methods (Figure 1), *R. gordoniae* was the strain with major capacity to interact with arsenite growing four times more than other strains. On the other hand, as much as *M. hydrocarbonoxydans* and *E. indicum* had an exponential growth in the first 24 hrs, which probably represents the arsenite oxidation step since both bacteria after the mentioned time begin the growth stationary phase. In this way, the ability to remove arsenic from contaminated water by the mentioned strains was corroborated.

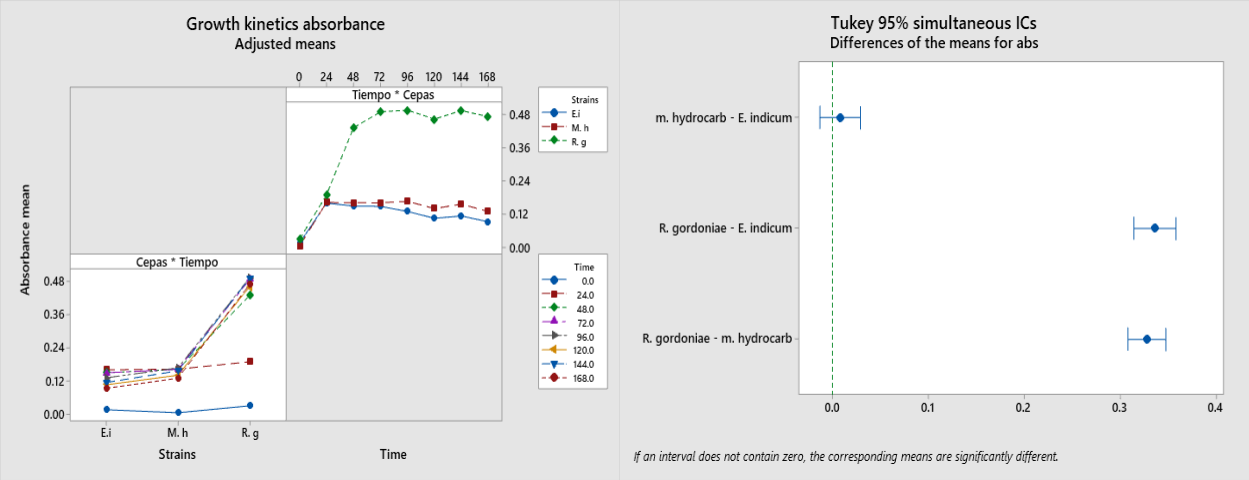


Figure 1. On the left, Growth Kinitics with arsenite.In color blue E. indicum, in red M. hydrocarbonoxydans and in green R. gordoniae.On the right, Tukey method of multiple comparisons, if an interval does not contain zero, the corresponding means are significantly different. Software MiniTab 2019.

***Table 1. Results of bacteria characterization.***

| | **Plasmids** | **aox genes** | **Production of PHB** |
|---|---|---|---|
| *R. gordoniae* | n.d | n.d | detected |
| *E. indicum* | n.d | aoxAB | n.d |
| *M. hydrocarbonoxydans* | n.d | aoxAB | n.d |

*(n.d) no detected.

## 5. CONCLUSIONS.

The strain *R. gordoniae* could have a different As(III) interaction mechanism that could differ from the ones that present redox reactions. This could be due the absence of aox genes. By means of the aox genes detection, it was proved that *E. indicum* and *M. hyudrocarbonoxydans* are As(III) oxidizer bacteria

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